**Response to Reviewers’ Comments**

**Reference number**: RSOS-241265

**Title of article**: Carcass size, not source or taxon, dictates breeding performance and carcass use in burying beetle

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Dear Dr. Bart Pannebakker,

Thank you for inviting us to submit a revised version of the manuscript. We greatly appreciate the valuable comments and feedback from you and the reviewers. We have carefully considered each comment and incorporated the suggestions. In particular, we have made the following major changes:

* Corrected the citation format issue and added several recent articles to the manuscript to better reflect the current status of IGP research.

Please also see the following section for our detailed point-by-point responses. All line numbers refer to the changes we made in the revised manuscript (with tracked changes). We believe that the revisions have further improved the quality of this manuscript, and we hope that it is now suitable for publication in *Royal Society Open Science*.

Sincerely,

Gen-Chang Hsu (submitting author)

Department of Entomology, Cornell University

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**Editor's comments**  
  
**Comment 1** > Thank you for submitting your work to Royal Society Open Science. Your work has now been reviewed by two reviewers. As you can see, both are positive and praise the quality of your science and of the readability of the manuscript, and I can only agree with them. Your work on the relation between carcass size, origin and taxon on the breeding success of *Nicrophorus nepalensis*, is a sound study which would fit Royal Society Open Science well. However, there a number of issues indicated by both reviewers that need to be revised before the paper can be accepted. Besides a list of minor issues, these concern some (small) concerns on the statistics, and a request by Reviewer 1 to extend the interpretation and discussion of the results. He provides some suggestions for papers which are a good starting point.

All in all, these are not big concerns, and I would like to encourage you to use the comments by the reviewers to further improve the paper. I am looking forward to seeing the revised version of your paper.

**Response 1** > Thanks for the positive feedback on our manuscript. We greatly appreciate the comments and suggestions from the reviewers and have now revised the manuscript accordingly. We also addressed the statistical concerns raised by the reviewers and expanded our discission on XXX as suggested by Reviewer 1. Please see our point-by-point responses in the following section.

**Reviewer 1's comments**

**Comment 1** > I commend the authors for this interesting study. However, the authors have missed many studies in the scientific literature that test the same hypotheses and provide additional detail for other species of burying beetles. I suggest the authors dig deeper into the literature and revise their interpretation and discussion. Below are specific points that should be addressed.

**Response 1** > Thanks for the feedback. We have now expanded the discussion on our results and incorporated the suggested references. Please see our response to Comment XXX for more details.

**Comment 2** > Line 28 and 49 Rather than just using the word "carcasses", you should specify "small vertebrate carcasses".

**Response 2** > Revised (Line XXX).

**Comment 3** > Lines 63-65 "Such cost-benefit trade-offs suggest that reproductive performance might not necessarily be greater on larger carcasses, yet no study has empirically examined whether there is an optimal carcass size for breeding." This is not true, several papers have addressed this issue in burying beetles. These papers have used single bout and lifetime reproductive success to evaluate optimal carcass size. See:

Belk, Mark C.; Meyers, Peter J.; Creighton, J. Curtis. 2021. Bigger is better sometimes: the interaction between body size and carcass size determines fitness, reproductive strategies, and senescence in two species of burying beetles. Diversity 2021, 13, 662. <https://doi.org/10.3390/d13120662>.

Quinby, BM, Belk, MC, Creighton, JC. Behavioral constraints on local adaptation and counter‐gradient variation: Implications for climate change. EcolEvol. 2020; 10: 6688– 6701. <https://doi.org/10.1002/ece3.6399>

Creighton, J.C., N.D. Heflin, and M.C. Belk.  2009.  Cost of reproduction, resource quality, and terminal investment in a burying beetle.  American Naturalist 174:673-684.

**Response 3** >

> Update the introduction and references.

**Comment 4** > Lines 66-78. This is a really important point and a novel part of the study. I suggest the authors enhance the discussion of this point, and I commend them for including it in the study.

**Response 4** > Thanks for the positive comment. We have modified the relevant paragraphs in the introduction section (Line XXX) and the discussion section (Line XXX) to strengthen our point on carcass source and taxa.

**Comment 5** > I notice that all of your experiments and analyses are based on single bouts of reproduction (presumably the first bout, although this should be explained). There is a difference between single bout and lifetime reproductive output. For example, in another species of burying beetle there is a clear difference between single bout reproductive success on 20 and 30 gram carcasses. However, lifetime reproductive output is equivalent on these two sizes. At the very least you should consider this difference in the discussion.

**Response 5** > Thanks for the suggestions. The study species *N. nepalensis* does reproduce multiple time throughout the life, and in our study, we recorded only the breeding outcomes during the first reproductive bout. We have now mentioned this in the methods section (Line XXX). As suggested, we also brought up the difference between single vs. lifetime reproductive output in the discussion section (Line XXX).

**Comment 6** > Line 231. Please provide an explanation for using a type II sums of squares approach. Usually, Type III sums of squares provide a more complete test of the hypothesis with multiple predictors.

**Response 6** > We used type II sums of squares in the ANOVA because it respects the principle of hierarchy (marginality). For example, in a model with two predictors A and B as well as the interaction A×B, it first tests the interaction term A×B (Y ~ A + B vs. Y ~ A + B + A×B) and drops A×B to test the main effect of A (Y ~ B vs. Y ~ A + B) and B (Y ~ A vs. Y ~ A + B).

On the other hand, type III sums of squares tests the main effect when all other terms are kept in the model. For example, for the same model above, it first tests the interaction term A×B (Y ~ A + B vs. Y ~ A + B + A×B), which is the same as type II sums of squares. However, it tests the main effect of A (Y ~ B + A×B vs. Y ~ A + B + A×B) and B (Y ~ A + A×B vs. Y ~ A + B + A×B) in the presence of A×B. The models Y ~ B + A×B and Y ~ A + A×B are meaningless because they contain the higher order term A×B without the lower-order terms A and B.

Besides the issue of marginality, studies have also shown that type II sums of squares generally has higher statistical power than type III sums of squares and therefore is more appropriate for testing main effects (Langsrud 2003, Smith & Cribbie 2014).

References:

Langsrud, Ø. (2003). ANOVA for unbalanced data: Use Type II instead of Type III sums of squares. *Statistics and computing*, *13*(2), 163-167.

Smith, C. E., & Cribbie, R. (2014). Factorial ANOVA with unbalanced data: a fresh look at the types of sums of squares. *Journal of Data Science*, *12*(3), 385-403.

**Comment 7** > Lines 341-342. I don't think this is an accurate statement. The citation is old, and the level of involvement of both parents varies among species. Do you have information from field data about *N. nepalensis* specifically?

**Response 7** > Based on the field data collected in a previous study, a group size of 2 is common in *N. nepalensis* (Fig. 2 in Liu et al. 2020). However, the group size can vary substantially across the elevations and with population densities. To avoid confusion, we have now removed the statement (Line XXX).

Reference:

Liu, M., Chan, S. F., Rubenstein, D. R., Sun, S. J., Chen, B. F., & Shen, S. F. (2020). Ecological transitions in grouping benefits explain the paradox of environmental quality and sociality. *The American Naturalist*, *195*(5), 818-832.

**Comment 8** > Line 352. See above comment. This is not the first time this has been shown.

**Response 8** > Revised (Line XXX).

**Comment 9** > Line 359-360. Not quite an accurate statement. The balance between size and number of offspring is more involved than simply carcass size. Many studies not cited here have addressed this relationship in burying beetles.

**Response 9** > Thanks for pointing this out. Yes, we agree that there are many factors besides carcass size that can influence the offspring quality-quantity relationship in burying beetles. In fact, we did not intend to conclude that carcass size is the “only” or the “most important” factor. Instead, our study adds to the understanding that carcass size, among many other factors addressed in previous studies, can shape this quality-quantity balance. We have modified our statement to avoid potential misunderstandings (Line XXX).

**Reviewer 2's comments**

**Comment 1** >

**Response 1** >

Reviewer: 2  
  
Comments to the Author(s)  
The authors examine the impacts of carcass size, origin (lab-reared vs wild), and taxon (mammal, bird, or reptile) on various breeding success metrics in captive experiments using a burying beetle (Nicrophorus nepalensis). The authors further test for nutritional differences among carrion, estimate optimal carcass size, and test for a trade-off between larval size and number in the context of carcass size, origin, and taxon. The authors find strong evidence for an optimal size of carcass under laboratory conditions and find little difference in reproductive success between carcass origin and taxon, the latter of which did not differ substantially in protein/fat content. Overall, the study provides important validation of other work that uses lab-reared carcasses in experiments with Nicrophorus, as well as interesting evidence for optimal carcass size and an influence of carcass size on life history strategies.  
  
Overall, the manuscript was very clear and well written. The experiments were well-designed and carefully executed with clear and appropriate statistics, plots, and well-balanced and reasonable interpretations. It was just a really nice, refreshing manuscript to read. I had a few comments, suggestions, and corrections, listed below.  
  
Main comment:  
  
1. The authors conduct many statistical tests using non-independent data, which can lead to some p-values that fall below 0.05 simply due to chance. Have you considered controlling for false discovery rates (e.g., Pike 2011, Methods in Ecology and Evolution)? Controlling for false discovery rates wouldn't change the main results of the paper, but would put some of the borderline p-values/results into better context.  
  
Minor comments:  
  
lines 61-65: What about additional costs of competitive interactions that vary with carcass size? For example, are large carcasses more likely to be found and eaten by vertebrates, thereby reducing fitness? How does microbial competition vary with carcass size and with different environmental temperatures? [I see this discussed on lines 344-350 - great!]  
  
lines 117-120: Were carcasses from the lab versus wild at similar levels of decomposition?  
  
lines 126-128: I really liked the controlled and paired design.  
  
lines 151-153: Isolating viscera and muscle seems to miss independent fat deposits (e.g., often well-developed between the skin and muscle on migratory birds). Why not estimate fat/protein composition of the entire carcass (after removing external hair/feathers)? Wouldn't the entire carcass (excluding bones) be a better representation of what the beetles use?  
  
line 208: What is carcass ID? If it relates to only one row of data per analysis, then why include it as a random effect?  
  
lines 219-220: "Dead larvae were excluded from the analysis." How often did larvae die in the experiment?  
  
lines 262-263 vs Figure 4f: "... although larvae feeding on wild bird carcasses tended to gain more weight compared to those feeding on wild mammals and reptiles (Fig. 4f)." -  
larval growth was highest for mammal carcasses, according to figure 4f  
  
lines 297-299: Was this evident in your study as well? Or would this only be evident if you use natural soil or conduct the experiments in nature?  
  
lines 304-307: An interesting idea, and consistent with some suggestions that Yarrowia (in Nicrophorus secretions) 'pre-digest' carrion for the young.  
  
lines 321-322: Again, I don't see this result in figure 4f.  
  
lines 346-347: I would think that temperature may further interact with carcass size and competitors to shift optimal carcass size in nature.  
  
lines 344-350: I really appreciated the discussion section acknowledging the potential for different optimal carcass sizes in nature, where other selective pressures (e.g., vertebrate scavengers) could play important roles.